CHAPTER 6
DISCUSSION

Isoproterenol (ISO) induced myocardial infarction is a standardized model to study the beneficial effects of many drugs and antioxidants on cardiac function. ISO, a β-adrenergic agonist and a synthetic catecholamine causes severe stress in the myocardium resulting in an infarct-like necrosis of heart muscle. It has been reported that the pathophysiological changes that take place following ISO induced myocardial infarction resembles the changes taking place after myocardial infarction in human beings (Manjula and Devi, 1993; Whealthy *et al.*, 1985). ISO is known to cause increased reactive oxygen species *via* auto-oxidation and subsequent oxidative stress which leads to myocardial necrosis (Bors *et al.*, 1978). Higher level of catecholamines depletes the energy reserve of cardiac muscle cells, leading to complex biochemical and structural changes that cause irreversible cellular damage and ultimately necrosis (Rona, 1985).

Cardiovascular disease is primarily caused by chronic deficiencies of vitamins and other essential nutrients with defined biochemical properties such as coenzymes, cellular energy carriers and antioxidants. Chronic depletion of these essential nutrients in endothelial and vascular smooth muscle cell impairs their ability to function properly (Divakar, 2002). Many dietary antioxidants and some non-nutrient based antioxidants from plants such as sulphur containing compounds in garlic, green tea, anthocyanins in red berries, lycopene in tomatoes, red and white wines from grape seeds are increasingly being recognized as potential health promoters in reducing the risk of cardiovascular disease (CVD) and atherosclerosis (Walker, 1996). The prophylactic and therapeutic effects of many plant foods and extracts in reducing CVD have been reviewed (Ames *et al.*, 1993). Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine owing to their non-addictive and non-toxic nature. Novel antioxidants may offer an effective and safe means of counteracting some of the problems and boosting the body's defense against free radicals and CVD. Regular ingestion of flavonoid-containing foods may protect against death from coronary artery disease

in men due to their strong free radicals scavenging activity (Hertog *et al.*, 1993; Ying, 1997).

Present study shows, a significant increase in heart/body weight ratio in ISO injected rats. Increased heart/body weight ratio indicates cardiac hypertrophy (i.e enlargement of heart) which may be due to ventricular stiffness, increased water content and extensive necrosis of cardiac muscle followed by invasion of the damaged tissue by inflammatory cells (Nirmala and Puvanakrishnan, 1996; Weber and Brilla, 1991). Increased generation of reactive oxygen species and oxidative stress is also implicated in the progression of cardiac hypertrophy and heart failure (Dhalla *et al.*, 2000; Choudhary *et al.*, 2006). Hypertrophy has also been characterized as a compensatory response to myocyte loss (Collins *et al.*, 1975).

Treatment of Vit. E alone and in combination with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly reduced the heart/body weight ratio which might be due to potent antioxidant activity of these combinations which reduced the stimulus for hypertrophy. However, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant effect on heart/body weight ratio. Addition of these antioxidants like GT, LYP and PGFE along with Vit.E give a beneficial effect rather that Vit.E alone.

Electrocardiograph (ECG)-abnormalities are the main criteria generally used for the definite diagnosis of MI. ST-segment elevation was observed in patient with acute myocardial ischemia (Peacock, 2007; Kela *et al.*,1980) and in ISO-induced MI in rat (Rajadurani and Prince, 2007a). Present study shows significant alterations in ECG patterns of ISO injected rats. The characteristic findings were reduction in R-R intervals, P wave intensity, QRS complex and elevation of ST segment, QT interval and heart rate. These alterations could be due to the consecutive loss of cell membrane potential in injured myocardium. The loss of

cell membrane function due to oxidative stress and elevation of ST segment is reported by several studies (Holland and Books, 1977; Kela *et al.*, 1980). Studies also suggest that the appearance of ST elevation and a long QT interval on ECG is associated with a high risk of MI and sudden cardiac death (Schwartz and Wolf, 1978; Moss, 1993). Increased ST segment reflects a potential difference in the boundary between ischemic and non ischemic zones and consequent loss of cell membrane functions in the regional ischemic myocardium (Kela *et al.*, 1980). ISO injected rats did not show statistically significant changes in heart rate which is supported by previous reports (Goyal *et al.*, 2010a; Goyal *et al.*, 2010b; Thippeswamy *et al.*, 2009). ISO caused myocardial damage was accompanied by a stress response in pituitary adrenal axis resulting in greater adrenal weight and adrenal hyperactivity which elevates circulating catecholamine levels (Wexler, 1978). This could account for the elevated heart rate in the present study.

Treatment of Vit. E alone and its combination with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) showed a protective effect against altered ECG pattern suggesting that these combinations could effectively prevent membrane potential. In this context Ithayarasi *et al* (1996) have reported that Vit.E treatment decreased ST elevation and prevented Q wave abnormality in ISO induced MI. Further it has been reported that GT extract prevented the ECG changes in doxorubicin induced cardiotoxicity (Patil and Balaraman, 2005). Treatment with LSFJ in ISO injected rats (LSFJ+ISO) shows a significant decrease in ST elevation. However, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show protective effect against altered ECG pattern compared to Vit.E alone treated group (Vit.E+ISO). Fard *et al.*, (2008) reported that the fruit of *Lagenaria siceraria* prevents ECG changes in doxorubicin induced cardiotoxicity. Addition of these antioxidants like GT, LYP and PGFE along with Vit.E produce a beneficial effect rather than Vit.E alone.

Acute ISO administration in rats and hamster produced a significant tachycardia associated with reduced aortic blood pressure thus indicating that relative myocardial ischemia due to mismatch between increased myocardial oxygen demand and reduced coronary blood supply (Chappel *et al.*, 1951; Rona *et al.*, 1959a and 1959b). In the present study ISO injected rats produced myocardial infarction which is evident by significant fall in systolic, diastolic and mean blood pressure. These changes in hemodynamic parameters indicated the activation of sympathetic nervous system. The decrease in blood pressure in the present study is in line with previous reports (Goyal *et al.*, 2009; 2010a; 2010b; Asdaq and Inamdar, 2009; Thippeswamy *et al.*, 2009). Further it has been reported that release of nitric oxide due to the activation of iNOS might be responsible for decrease in blood pressure and agents that inhibit the induction or the activity of iNOS are able to prevent or reverse the hypotension (Szabo, 1995). Results of present study also showed a significant increase in the NO level.

Supplementation of Vit.E alone and co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly attenuated these changes in hemodynamic parameters evidenced by the improvement in systolic, diastolic and mean blood pressure. This improvement in hemodynamic parameters might be due to potent antioxidant activities of these drugs which decreased NO production from iNOS. However co- administration of Vit.E with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant improvement in hemodynamic changes compared to Vit.E+ISO treated rats.

Elevated levels of cardiac marker enzymes such as transaminases, ALP, CK-MB and LDH are the diagnostic indicators of MI (Hearse *et al.*, 1979). The release of cellular enzymes reflects the alterations in membrane integrity or permeability as a result of β-adrenergic stimulation. AST and ALT are the enzymes associated with the conversion of aminoacids to ketoacids and are used as marker enzymes to indicate tissue damage. An increase in ALP in serum reflects the pathological

alterations in biliary flow (Ong and Khoo, 2000). CK-MB is commonly regarded as an indicator of cell death because it requires both disintegration of contractile apparatus and leaky plasma membrane (Mair *et al.*, 1944). Thus, increased cardio specific CK-MB indicates myocardial damage. LDH is a cytosolic enzyme involved in glycolysis and responsible for the interconversion of pyruvate and lactate with concomitant interconversion of NADH and $NADH^+$. Detection of elevated concentration of these enzymes becomes a definitive diagnostic criterion for cardiac toxicity and location of tissue damage (Jaffe *et al.*, 1996). Oxidative stress causes membrane injury of the myocardium which leads to the release of these marker enzymes and compounds having antioxidant properties significantly prevent this leakage of enzymes from damage myocardium (Ebenezar *et al.*, 2003).

In the present study, ISO injected rats showed a significant elevation in the levels of these diagnostic marker enzymes (AST, ALT, ALP, LDH and CK-MB). Moreover, elevated levels of these enzymes are an indicator of the severity of ISO-induced myocardial membrane necrosis, which is in line with an earlier report (Panda and Naik, 2008; Rajadurai and Prince, 2006). Increased activities of these marker enzymes in the serum are indicative of cellular damage and loss of functional integrity of cell membrane (Bhakta *et al.*, 1999). The free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to loss of structural and functional integrity of the membrane.

A significant increase was observed in the activities of CK-MB, LDH, ALP and transaminases in ISO injected rats is due to the leakage of enzymes from the heart as a result of oxidative stress and necrosis induced by ISO (Manjula *et al.*, 1993). The myocardial membrane becomes permeable or may rupture, due to deficient oxygen supply or glucose, thereby resulting in the leakage of these enzymes which find their way into the blood stream, thus increasing their concentration in the serum (Wexler and Kittinger, 1963).

Treatment of Vit.E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly decreased the elevated levels of serum marker enzymes towards normal. This reduction in marker enzyme levels could be due to the potent action of these combinations which prevents membrane integrity and/or permeability thereby restricting the leakage of these enzymes from the myocardium. In this context it has been reported that treatment with Vit.E reduced the elevated levels of cardiac marker enzymes in ISO injected rats (Ithayarasi *et al.*, 1996; Nandave *et al.*, 2007). Further it has been reported that treatment with Green tea prevents the elevation of serum marker enzymes in doxorubicin induced cardiotoxicity (Patil and Balaraman, 2005) and ISO induced MI in rats (Devika and Prince, 2008a, Upaganlawar *et al.*, 2009).

Co-administration of Vit.E with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant improvement in cardiac marker enzymes as compared to Vit.E alone treated group (Vit.E+ISO). However, treatment with LSFJ alone in ISO injected rats (LSFJ+ISO) significantly decreased cardiac marker enzymes. In this context it has been reported that *Lagenaria siceraria* fruit prevents elevation of serum marker enzymes in doxorubicin induced cardiotoxicity (Fard *et al.*, 2008) and CCl_4 induced hepatotoxicity (Deshpande *et al.*, 2008).

In the present study a significant increase in serum uric acid levels and a decrease in total protein levels was observed in ISO-injected rats which is in line with the previous reports (Rajdurani and Prince, 2007; Devika and Prince, 2007). Increased uric acid levels are commonly considered as a consequence rather than a cause of CVD. Large cohort studies have shown that uric acid is an important independent risk factor for cardiovascular mortality and in the development of MI (Niskanen *et al.*, 2004; Fang and Alderman, 2000; Weir *et al.*, 2003). In hypoxic tissue, ATP depletion occurs causing accumulation of hypoxanthine. When tissues are disturbed, the enzyme xanthine dehydrogenase is converted to xanthine oxidase

by the oxidation of essential-SH-groups. Xanthine oxidase catalyses the conversion of hypoxanthine to xanthine, uric acid and superoxide. The dehydrogenase to oxidase conversion occurs in ischemic or hypoxic tissue (Mc Cord, 1988). This could be one of the reasons for the elevated levels of serum uric acid in ISO-injected rats. Studies also show that serum uric acid concentrations are higher in patients with established coronary heart disease compared with healthy control (Torun *et al.*, 1998). A decrease in the level of serum total proteins in ISO-injected rats could be due to increased free radical production by ISO. Drugs having antioxidant properties have been showed to reduce elevated uric acid levels and increase serum protein levels (Rajdurani and Prince, 2007).

Treatment of rats with Vit. E and its combination with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+GT+ISO) significantly decreased the uric acid levels and increased serum protein levels. This effect might be due to the potent antioxidant activity of these combinations which prevent the oxidation of 'SH' group and thereby uric acid formation. Co-administration of Vit.E and LSFJ did not show significant improvement in serum uric acid and protein levels as compared to Vit.E alone treated rats. In this context vitamin E has been reported to reduce platelet xanthine oxidase activity in patients of MI owing to its antioxidant activity (Raghuvanshi *et al.*, 2005) and lycopene reduces the xanthine oxidase activity in plasma and muscle tissue of rats (Chieh-Chung *et al.*, 2005). Addition of GT, LYP or PGFE along with Vit.E shows beneficial effects rather that Vit.E alone in preventing cardiac marker enzymes.

Myocardial injury can be differentiated from other types of tissue damage since, the LDH isoenzyme begins to rise in 12-24h following myocardial injury, peak in 2-3 days (Jaffe *et al.*, 1996). For better specificity of cardiac injury measurement of LDH isoenzymes is necessary, since a non specific increase of total LDH in serum occur following tissue damage. LDH is a cytosolic enzyme exists in five different isoforms (LDH1-LDH5). LDH1 predominates in cardiac tissues. Variation in the

serum LDH isoenzyme pattern can be considered as definitive diagnostic criterion for assessing the myocardial damage as the rate of appearance and disappearance of LDH in the blood indicates the size of infarction (Trofimov *et al.,* 1975). In the present study, an increase in LDH1 and LDH2 isoenzyme bands in ISO injected rats were observed which is supported by previous findings (Priscilla and Prince, 2009; Karthikeyan *et al.*, 2007).

Co-administration of Vit. E with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly reduced the intensity of LDH1 and LDH2 isoenzyme bands. The reduction in the intensity of LDH1 isoenzyme by these combinations could be due to potent antioxidant activities, which may prevent the leakage of LDH enzymes from the myocardium. Treatment of LSFJ in ISO injected rats (LSFJ+ISO) showed significant decrease in the intensity of LDH1 isoenzyme. However, co-administration of Vit.E and LSFJ did not produce further reduction in the intensity of LDH1 isoenzyme. Addition of GT, LYP or PGFE along with Vit.E shows beneficial effects in reducing the intensity of LDH1 isoenzyme rather than Vit.E alone.

The main target for reactive oxygen species (ROS) appears to be polyunsaturated fatty acids (PUFA), which is the precursor for lipid peroxidation reaction. Malondialdehyde is a major lipid peroxidation end product; increased malondialdehyde content may contribute to increased generation of free radicals, altered membrane structure and decreased activities of antioxidant enzymes (Jayachandran et al., 2009). Increased levels of lipid peroxidation products can also injure blood vessels, increased adherence and aggregation of platelets to the injured sites (Sathish *et al.*, 2003a). In the present study a significant increase in the lipid peroxidation products following ISO injection was observed. Elevation of lipid peroxides in ISO injected rats could be attributed to the accumulation of lipids in the heart and the irreversible damage to myocardial membranes (Sathish *et al.*, 2003b).

ROS are generated from the leakage of electrons into oxygen from various systems in our body and the endogenous antioxidant enzyme defense is a very important source to neutralize the oxygen free radical mediated tissue injury. Oxidative stress in tissues resulted in increased production of ROS and depletion of the antioxidants in the defence system, thereby causing an imbalance between prooxidants and antioxidants (Mallet and Sun, 2003). Decreased in the activities of antioxidant enzymes is in close relationship with induction of LPO (Jagetia *et al*, 2003). It has been reported that, auto-oxidation of ISO produces quinones, which react with oxygen to produce superoxide anions and hydrogen peroxide, leading to oxidative stress and depletion of the endogenous antioxidant system (Adameova et al., 2009).

In the present study a significant decreased in the activities of GSH, GPX, GST, SOD and CAT was observed in ISO injected rats as compared to control groups, suggesting an enhanced oxidative stress after ISO injection. Present results are in agreement with previous reports (Rajdurani and Prince, 2006; Nirmala and Puvankrishnan, 1996; Saravanan and Prakash, 2004). GSH is an abundant and ubiquitous antioxidant, which exerts its function by reacting with superoxide radical, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione and other disulfides (Meister, 1988). The decreased levels of GSH in ISO injected rats may be due to increased generation of ROS. Activities of GSH-dependant antioxidant enzymes such as GPx and GST were significantly reduced in ISO injected groups, reflecting an increased oxidative stress in MI (Nirmala and Puvankrishnan, 1996). GPx is a major enzymatic mechanism for the disposal of hydrogen peroxides and lipid peroxide thereby offering protection to the cellular and subcellular membranes (Mallet and Sun, 2003). Depression in the activity of this enzyme may lead to the intracellular accumulation of peroxides. GST is a member of complex supergene encoded family of detoxification enzymes, acts like peroxidase and removes the stable peroxides from the system resulting in reduction of peroxide induced damage (Jagetia *et al.*, 2004). The unavability of

GSH may decrease the activity of GPx and GST in ISO injected rats (Saravanan and Prakash, 2004).

SOD acts by protecting the cell from oxidative damage by converting superoxide radical into hydrogen peroxide. Hydrogen peroxide is further metabolized by catalase to water and molecular oxygen (Marczin *et al.*, 2003). Reduction in the activity of SOD and CAT in the present study may be due to the increased generation of super oxide and hydrogen peroxide radicals, which in turn leads to inactivation of these enzymes (Searle and Wilson, 1980). Vit. E is a major lipid soluble non-enzymatic antioxidant present in cell membranes and lipoproteins that protects against oxidative modification (Prabhu et al., 2006). The observed decrease in the Vitamin E level, in the present study, might be due to increased utilization for the neutralization of ISO mediated free radicals and lipid peroxidation.

Treatment of Vit. E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly decreased lipid peroxidation and thereby reduced oxidative stress generated by ISO. These combinations also significantly increased the biomarkers of oxidative stress and endogenous vitamin E levels. Several reports are available in this context that antioxidants in combination shows better effects rather than alone in attenuating oxidative stress (Yogeeta *et al.*, 2006; Qing *et al.*, 2006; Punithavathi and Prince, 2010; Upaganlawar *et al.*, 2009; Hagar 2002; Limpens *et al.*, 2006). Co-administration of Vit.E and LSFJ did not show beneficial effects in the improvement of Lipid peroxidation and the activities of antioxidant enzymes. However treatment of LSFJ alone in ISO injected rats (LSFJ+ISO) showed significant decrease in lipid peroxidation and an improvement in GSH level. Addition of GT, LYP or PGFE along with Vit.E shows beneficial effects rather than Vit.E alone in attenuating LPO and oxidative stress induced by ISO.

Vitamin E is the most important lipid soluble radical scavenger in membranes and plasma. It was demonstrated that α-tocopherol donates the hydrogen atom to chain propogating lipid peroxyl radicals, giving rise to a relatively stable α-tocopheroxyl radical (Witting, 1980). This could be one of the reasons for its antioxidant activity which prevents oxidative stress induced by ISO in the present study. Nandave *et al.*, (2007) have reported that administration of Vit. E prevents ISO induced oxidative stress and maintained lipid peroxidation and biomarkers of oxidative stress due to its potent antioxidant activity.

Green tea is rich in catechins. Catechins have gallate moiety esterified at the 3rd position of the C ring, the potent free radical scavenging activity of green tea is attributed to the presence of the C ring of gallate group. Further it was reported that more the hydroxyl groups in the catechin, more the free radical scavenging activity (Zhao *et al.*, 2001). The presences of hydroxyl groups in the structure of GT make it hydrophilic and easier accesses to the area where the lipids free radicals are generated, facilitating its reaction with lipid free radicals (Wang *et al.*, 2009). EGCG has been reported to prevent ISO induced MI and GT prevents doxorubicin induced cardiotoxicity (Devika and Prince, 2007; Patil and Balaraman, 2005). GT in the present study reduced lipid peroxidation and prevents oxidative stress, which might be due to its interaction with peroxyl radical (Katiyar *et al.*, 2000; Hong *et al.*, 2001), superoxide radical and hydrogen peroxide produced by ISO (Devika and Prince, 2007). Zhou et al, (2005) have reported that polyphenols donate hydrogen atoms to tocopheryl radicals and enhance the antioxidant efficiency of alpha tocopherol and eliminates the so called tocopherol mediated peroxidation. This may possibly be the reason for the beneficial effect of green tea and vitamin E in attenuating oxidative stress in the present study.

Vit.E and LYP both are lipid soluble antioxidants. LYP is well known scavenger of singlet oxygen and other excited species. LYP is reported to prevent 7-ketocholesterol induced oxidative stress, myocardial ischemia-reperfusion injury

and cisplatin induced oxidative stress owing to its antioxidant activity (Palozza *et al.*, 2010; Bansal *et al.*, 2006; Atessahin *et al.*, 2005). Carotenoids are reported to remove peroxyl radicals most efficiently at low oxygen tension (Kennedy and Leibe, 1992). During singlet oxygen quenching, energy is transferred from singlet oxygen to lycopene molecule, converting it to the energy rich triplet state (Wertz *et al.*, 2004). Further it was reported that the synergistic effects of LYP and Vit. E in combination may be due to the regeneration of Vitamin E from its α-tocopheroxyl radical by lycopene (Palozza and Krinsky, 1992). This may possibly be the reason for the beneficial effect of lycopene and vitamin E in attenuating oxidative stress in the present study.

Pomegranates are predominantly rich in polyphenols, including primarily hydrolysable ellagitannins, anthocyanins and other polyphenols. Ellagitannins found in the outer part of the fruit are largely responsible for the antioxidant activity of the pomegranate (Gil *et al.*, 2000). It has been demonstrated that one of the ellagitannins, punicalagins (punicalagin anomers A and B) are responsible for over 50% of the antioxidant activity of the pomegranate (Gil *et al.*, 2000). Pomegranate is reported to prevent ferric nitrilotriacetate (Fe-NTA) and CCl_4 induced hepatotoxicity evidenced by mitigating hepatic lipid peroxidation and antioxidant enzymes (Kaur *et al.*, 2006; Kotamballi *et al.*, 2002). The presence of different polyphenolic compounds in pomegranate could be responsible for its exhibited potent antioxidant activity in the present study.

In the present study, co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not produce beneficial effects in attenuating lipid peroxidation and oxidative stress compared to Vit.E alone treated group (Vit.E+ISO). However rats treated with LSFJ alone in ISO injected rats (LSFJ+ISO) showed significant reduction in lipid peroxidation and GSH levels which might be due to the presence of polyphenols. In this context Fard *et al* (2008) and Deshpande *et al* (2008) reported that administration of *Lagenaria siceraria* fruit

extract and *Lagenaria siceraria* epicarp extract prevented biomarkers of oxidative stress in doxororubicin induced cardiotoxicity and CCl_4 induced hepatotoxicity owing to is polyphenolic contents. LSFJ also showed *in vitro* antioxidant activity against DPPH radical scavenging assay (Deshpande *et al.*, 2007).

In vitro studies of Vit.E, GT, LYP and PGFE reveals a strong antioxidant activity against DPPH radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay and inhibition of lipid peroxidation in dose dependent manner. LSFJ shows mild antioxidant activity in scavenging DPPH radical, superoxide radical and inhibition of lipid peroxidation. However it did not show nitric oxide radical scavenging activity at the same dose. This result supports the *in vivo* antioxidant activity of the drugs in the present study.

ATPases play a significant role in the contraction and relaxation of the cardiac muscle by maintaining the normal ion levels inside the myocyte. In the present study ISO injected rats showed a significant decrease in Na^+/K^+ ATPase and Mg^{2+} ATPase activity and correspondingly Ca^{2+}ATPase activity was increased. The changes in the ATPase activity are in line with the previous reports (Devika and Prince, 2007; Devika and Prince, 2008; Jayachandran *et al.*, 2009). Na^+/K^+ ATPase is a lipid dependent enzyme containing sulfhydryl (-SH) group. The most important mechanism of free radical damage to enzymes is by oxidation of SH group within the enzyme (Dixon *et al.*, 1990). Oxidants are known to initiate lipid peroxidation, and thus destroy phospholipids which are required for the normal activity of membrane bound protein. The present study also shows increased lipid peroxidation and decreased in the phospholipids content in myocardium following ISO injection. This could be the reason for alteration in the ATPase activity. Increased concentrations of FFA and cholesterol in the myocardium resulted in non-competitive inhibition of many enzymes such as Na^+/K^+ ATPase, thereby leading to increased accumulation of Na^+ ions in ISO injected rats (Ahmed and Thomas, 1971).

Result of the present study also displayed enhanced activity of Ca^{2+} ATPase; it may be due to the activation of adenylate cyclase activity. ISO induced MI has been reported to enhance adenylate cyclase activity, resulting in increased formation of cAMP (Subhash *et al.*, 1978; Martorana. 1971). During beta adrenergic stimulation, cAMP phosphorylates several sites on the C-terminal chains of the calcium channel opening (Varadi *et al.*, 1995). This may be one of the reasons for enhanced Ca^{2+}ATPase activity and thereby increasing Ca^{2+} contents. Calcium overload in the myocardial cells during ischemia activates the Ca^{2+} dependant ATPase of the membrane depleting high energy phosphate stores, thereby indirectly inhibiting Na^+ and K^+ transport and inactivation of Na^+/K^+ATPase (Rajdurani and Prince, 2007b).

In the cell, ATPases are intimately associated with the plasma membrane and participate in the energy dependent transport of sodium, potassium, magnesium and calcium translocation (Mourelle and Franco, 1991). An increase in sodium and calcium along with decrease in potassium were observed in ISO injected rats. As discussed in above paragraph, the alteration of ATPase brings about the change in the concentration of electrolytes. Increased concentration of sodium might be due to decrease in Na^+/K^+ ATPase (Jennings *et al.*, 1986). Depletion of ATP by ISO leads to opening of K^+ channel leading to the decrease in K^+ ions in the myocardial tissue. Increased levels of intracellular Na^+ also operate to depress Ca^{2+} effect and augment Ca^{2+} influx.

In the present study, treatment of Vit.E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly increased the activities of Na^+/K^+ ATPase and Mg^{2+} ATPase and decreased the activity of Ca^{2+} ATPase. These combinations also prevent the alteration in Na^+, K^+ and Ca^{2+} levels. The protective effects of these combinations could be due to the prevention of 'SH' group from oxidative damage through the inhibition of peroxidation of membrane lipids indicating the

membrane stabilizing effects of these combinations. In this context EGCG is reported to protect membrane bound ATPase in ISO injected rats (Devika and Prince, 2008) and GT protects ATPase levels in doxorubicin induced cardiotoxicity in rats (Patil and Balaraman, 2005). Further the present study also shows the beneficial effect of Vit.E along with GT, LYP or PGFE in reducing lipid peroxidation. This might be the reason for better effects of Vit.E along with GT, LYP or PGFE in attenuating membrane bound ATPases and electrolyte levels than Vit.E alone. Treatment of Vit.E along with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) slightly alters the ATPase activity and electrolyte levels but was found to be statistically non-significant. Addition of GT, LYP or PGFE along with Vit.E shows beneficial effects rather than Vit.E alone.

Lipid plays an important role in cardiovascular diseases, by modifying the composition, structure and stability of cellular membranes. Altered lipid metabolism is considered to accelerate the development of atherosclerosis which is a major risk factor in MI (Onyeneke *et al.*, 2007). High levels of circulating cholesterol and its accumulation in heart tissue is well associated with cardiovascular damage (Slater and White, 1996). ISO induces free radicals (Singal *et al.*, 1983), which may cause cellular cholesterol accumulation, by (i) increasing cholesterol biosynthesis and its esterification, (ii) decreasing cholesterol ester hydrolysis and (iii) reducing cholesterol efflux (Gesquiere *et al.*, 1999).

Mathew *et al*, (1981) reported that hypertriglyceridemia is a prominent feature of ISO induced cardiovascular disturbances. It also increases the LDL cholesterol concentration in the blood, which in turn leads to the build up of harmful deposits in the arteries, thus favouring coronary heart disease (Goldstein *et al.*, 1979). High levels of serum triglyceride have been strongly linked with low serum HDL cholesterol and these low HDL levels may contribute to increased risk for CVD (Assmann *et al.*, 1986; Avogaro *et al.*, 1991). The observed pattern of change in cholesterol, triglyceride, phospholipids and free fatty acids in the serum and heart

of ISO injected rats agrees with the previous reports (Karthikeyan *et al.*, 2007, Nair and Devi, 2006). Yeagle (1985) reported that changes in membrane cholesterol content affect its fluidity, permeability to ions, activities of membrane-bound enzymes and increased degradation of phospholipids. A significant increase in free fatty acids and a decrease in phospholipids content in the heart of ISO-injected rats might be due to the breakdown of membrane phospholipids. The decline in the cardiac phospholipid content with a concomitant increase in the serum can be due to ISO mediated peroxidation of unsaturated membrane lipids in biomembranes and tissues causing the leakage of these lipids into circulation (Sathish *et al.*, 2003). Also, the increased peroxidation of membrane phospholipids releases free fatty acids by the action of phospholipase A_2 (Chein *et al.*, 1980). Increased cardiac lipid peroxidation is in concert with altered PL and FFA levels in ISO injected rats observed herein.

Changes in the serum lipids are controlled by those enzymes which are responsible for lipid metabolism. In the present study ISO injected rats showed a significant decrease in cardiac LCAT and LPL activities and a significant increase in the activity of CES. Lipoprotein lipase (LPL) is the crucial enzyme in the metabolism of triglyceride rich lipoproteins. Elevated triglyceride rich lipoprotein levels may not only promote a more rapid progression of atherosclerosis, but could also lead to myocardial ischemia (Nordis and Mack, 1995). In the present study hypertriglyceridemia observed in ISO injected rats is due to decreased activity of LPL in the myocardium resulting in decreased uptake of TG from the circulation. Lecithin: cholesterol acyl transferase (LCAT) is a serum enzyme that esterifies free cholesterol, primarily at the surface of the HDL particle after which the cholesteryl ester molecules migrate to the inner core of this lipoprotein (Onyeneke *et al.*, 2007). Through this action, LCAT plays a key role in the maturation of HDL particles. In humans, almost all serum cholesteryl esters are formed by the activity of LCAT and individuals devoid of this enzyme show abnormal plasma lipoproteins (Glomset *et al.*, 1970; Nordby and Norum, 1975).

Cholesterol esterification in the tissue is reported to be mediated through CES. The accumulation of ester cholesterol observed in ISO injected rats might be due to oxidative stress mediated increased activity of CES in the heart during MI (Yogeeta *et al.*, 2006).

Treatment of Vit. E alone and its combination with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) shows significant protection against altered lipid profile and lipid metabolizing enzymes. This improvement in serum and tissue lipid profile strongly supports the potent lipid lowering and antioxidant activities of these drugs. Co-administration of Vit.E with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show significant protective effects against altered lipid profile and lipid metabolizing enzymes activities compared to Vit.E alone treated group (Vit.E+ISO). The present study also showed significant reduction in the level of lipid peroxidation by these combinations. This could be one of the reasons for the prevention of altered lipid profile and lipid metabolizing enzymes by these drugs. Further a significant increase in the level of HDL in rats treated with Vit.E and its combination with GT, LYP or PGFE (Vit.E +ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO, Vit.E+PGFE+ISO) support the increased cardiac LCAT activity. Addition of GT, LYP or PGFE along with Vit.E shows beneficial effects rather than Vit.E alone.

In this context Vit. E has been reported to reduce lipid peroxidation formation from unsaturated fatty acids by inhibiting phospholipase A_2 and lipoxygenase activity in ISO injected rats (Yoshihara and Watanabe, 1990). This could be the reason for improving the lipid profile in the present study.

Green tea is particularly rich in epigallocatechin, a powerful antioxidant, which exerts a protective effect against LDL oxidation (Pearson *et al.*, 1998). It is known that the oxidatively modified LDL has a central role in atherosclerosis. The significant hypocholesterolemic effect of green tea is attributed to a reduction in

cholesterol absorption and to an increased excretion of biliary acids, cholesterol and also the inhibition of cholesterol synthesis in liver (Hasegawa *et al.*, 2003). Devika and Prince (2008) reported that treatment with Epigallocatechin gallate (E GCG) prevents mitochondria lipid profile by interacting with peroxy radicals and inhibiting lipid peroxidation. This might be the reasons for better lipid lowering activity of Vit.E with GT in the present study.

Lycopene as a lipid soluble antioxidant proved to prevent lipid peroxidation and thereby phospholipids degradation in the present study. Recently it has been reported that lycopene from tomato paste produced significant improvement in lipid profile and lower atherogenic index in hyperlipidemic rats and high fat diet fed rabbit (Ibrahim *et al.*, 2008, HU *et al.*, 2008). Fuhrman *et al* (1997) showed that the addition of lycopene to macrophage cell lines decreased cholesterol synthesis and increased LDL receptors, due to its non antioxidant function.

Esmaillzadeh *et al*, (2006) reported that Pomegranate juice decreased cholesterol absorption and increased its fecal excretion. It also showed beneficial effect on lipid metabolizing enzymes and significantly maintained LDL and HDL cholesterol in hyperlipidemic patients (Aviram *et al.*, 2000). The presence of different chemical constituents might be the responsible for the better lipid lowering effects of Vit.E along with PGFE in the present study.

LSFE shows significant lipid lowering activity in the present study which might be due to the presence of plant sterols (campesterol, fucosterol, etc.) and fixed oil in the fruit, the latter is considered as a good source of mono, poly unsaturated fatty acids and cardiac aglycones. Ghule *et al.*, (2006) showed the presence of sterols, fixed oil, cardiotonic aglycones, flavonoids, saponins and polyphenolics in different extracts of LSF. Plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of steroids resulting in decreased body lipids (Guimaraes *et al.*, 2000). Saponins are also reported to bind in intestinal lumen

with cholesterol, decrease its absorption, increase its fecal excretion and increase the LPL activity (Guimaraes *et al.*, 2000; Sidhu and Oakenful, 1986; 1990). Ghule *et al* (2006; 2009) reported that LSFE improved lipid profile in triton and high fat diet induced hyperlipidemic rats. Further, treatment of LSFJ decreased lipid peroxidation in the present study, which supports the lipid lowering activity of LSFJ.

In the present study ISO injected rats showed a significant rise in tissue nitrite levels which indicate increased NO production and thereby reactive nitrogen species (RNS). Mauricio *et al* (2006) have reported an 80–90% increase in nitrites and nitrates formation after ISO injection. During the acute phase of MI inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production increases in the heart (Pinto *et al.*, 2007). Dianyuan *et al* (2006) reported that β-AR stimulation upregulated iNOS and significantly increased production of NO, which created nitrosative stress and generated the powerful oxidant molecule peroxynitrite (ONOO-). iNOS can be induced by proinflammatory substances, such as cytokines and lipopolysaccharide (LPS) or events, such as ischemia, stroke, trauma and infection (Kelly *et al.*, 1996). The role of iNOS in myocardial ischemia remains controversial and seems paradoxical. Several studies showed that induction of iNOS contributed to myocardial ischemia/reperfusion injury possibly through the generation of nitrosative stress (Feng *et al.*, 2001; Sam *et al.*, 2001; Li *et al.*, 2006) in contrast, the study also showed that iNOS protected the heart through inducing myocardial preconditioning (Guo *et al.*, 1999).

Oxidative stress has been implicated in structural abnormalities of HF, causing loss of contractile function (Feng *et al.*, 2001). These detrimental effects may be due to persistent high amounts of NO reacting with superoxide anion, generating the highly reactive oxidant $OONO^-$ (Dianyuan *et al.*, 2006). *In vitro* studies have shown that iNOS not only produces NO but is also capable of generating superoxide independently of NO (Xia and Zweier, 1997). Superoxide leads to the

formation of H_2O_2, which may also be toxic (Chen *et al.*, 2005, Hare, 2004). It is important to point out that H_2O_2 and its oxidizing metabolites can promote lipid peroxidation (Pinto *et al.*, 2007). Drugs having potent antioxidant activity reduces the expression of iNOS and thereby nitrosative stress in myocardial injury by reducing the formation of superoxide radical and thereby peroxynitrites formation (Ribeiro *et al.*, 2009; Zhou *et al.*, 2006).

In the present study, treatment of Vit. E in ISO injected rats (Vit.E+ISO) reduced the nitrite formation but it was found to be non-significant. However, co administration of Vit.E along with GT or LYP in ISO injected rats (Vit.E+GT+ISO or Vit.E+LYP+ISO) significantly reduced the nitrite formation thus reduces not only NO production but also formation of superoxide, in turn reducing generation of H_2O_2 and nitrosative stress. *In vitro* study shows the superoxide and nitric oxide scavenging activity of GT which is due to the presence of tannins and gallate moiety in the structure (Yokozawa and Nakagawa, 2002; Prince and Rajdurani, 2007). GT also modulate iNOS expression in hypoxia/reoxygenation in cultured rat cardiomyocytes owing to its antioxidant property (Agnetti *et al.*, 2005). Further LYP is reported to inhibit nitrite production in microglia stimulated by lipopolysacceride (Hsiao *et al.*, 2004).

Co-administration of Vit.E and PGFE or LSFJ in ISO injected rats (Vit.E+PGFE+ISO or Vit.E+LSFJ+ISO) did not produce protective effects in decreasing nitrite levels compared to Vit.E alone. In this context pomegranate juice was reported to inhibit iNOS expression and oxidative stress in hyperoxaluria-induced oxidative stress and stone formation in rat kidneys (Ilbey *et al.*, 2009). Treatment with LSFJ did not produce any significant effects on nitrite levels in the present study. Both *in vitro* and *in vivo* experiments indicated weak antioxidant activity, in scavenging superoxide and nitric oxide, of this drug. Addition of GT or LYP along with Vit.E shows beneficial effects rather than Vit.E alone in reducing tissue nitrite levels.

A growing body of evidence suggests that systemic inflammation plays a key role in the pathogenesis of CVD. C-reactive protein (CRP) is one of the major acute phase reactants secreted by the liver in response to increased levels of inflammatory cytokines such as interleukin-6 and interleukin-1β (Calabro *et al.*, 2003; Libby *et al.*, 2002). Present study showed significant increase in CRP level in ISO injected rats suggested an involvement of inflammatory process during ISO induced MI. High concentrations of CRP have been used as a sensitive predictor of acute cardiovascular events compared with other widely used biomarkers such as total and LDL cholesterol (Ridker *et al.*, 2000; Verma *et al.*, 2006). Several population-based observational studies have reported that serum CRP concentrations are inversely associated with dietary intake of fruits, vegetables and tea, which are rich in polyphenolic antioxidants such as flavonoids (Chun *et al.*, 2007; Chun *et al.*, 2005). One proposed mechanism for the benefit of dietary flavonoids is the antioxidant properties (Knekt *et al.*, 2002; Robak, 1988). These polyphenols are effective scavengers of reactive oxygen species and can inhibit lipid peroxidation through chelation of transition metal ions or their chain breaking antioxidant activity (Leopoldini *et al.*, 2006; Acker *et al.*, 2000). These properties suggest that flavonoids might prevent LDL oxidation, an early key inflammatory event in the development of atherosclerosis (Lind, 2003). Oxidative stress leads to activation of nuclear transcription factor-kappa B (NF-kB) and DNA fragmentation. As a result, the inflammatory cascade is triggered and CRP is subsequently produced (Wanner and Metzger, 2002). Accumulation of inflammatory cells in the cardiac myocytes after ISO injection have been reported by Saad *et al* (2001) which strongly supports the present results.

In the present study administration of Vit. E alone and in combination with GT, LYP or PGFE in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly reduced the elevated CRP levels towards normal indicating potent anti-inflammatory and antioxidant activity of these combinations. Further, the present study shows significant reduction in the

elevated level of LDL and biomarkers of oxidative stress after treatment with these combinations. This might be the probable reason for reducing the level of CRP by these combinations. As the development of inflammatory process is correlated with the release of CRP, significant reduction in their release was observed in the present study provides biochemical evidence for the anti-inflammatory properties of these combinations (Vit.E+GT+ISO, Vit.E+LYP+ISO and Vit.E+PGFE+ISO).

In this context, Peter (2003) has reported that there is a significant inverse linear relationship between concentrations of CRP and plasma concentrations of the antioxidants lycopene, beta-carotene, cryptoxanthin and retinol. Pomegranate is reported to have inhibitory effects on renal tubular cell injury and oxidative stress caused by oxalate crystals by reducing ROS, iNOS and NF-kB expression in hyperoxaluria-induced oxidative stress and stone formation in rat kidneys (Ilbey *et al.*, 2009). Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not produce significant effect on CRP level compared to Vit.E alone.

It was noticed earlier that the neutrophils, a major source of free radicals, characteristically invaded the myocardial tissue during ischemia. Myocardial cell injury is related to the activation of polymorphonuclear neutrophils from which myloperoxidase (a heme enzyme) is secreted (Buffon *et al.*, 2002). In the present study, cardiac MPO activity was increased in ISO-injected rats. This is consistent with results from a previous study and indicates an acute inflammation and leukocyte accumulation in heart tissues of the ISO-injected animals (Senthil *et al.*, 2007). MPO catalyzes the formation of hypochlorous acid (HOCl), a powerful oxidant derived from chloride ions and hydrogen peroxide (H_2O_2). HOCl may then interact with other small molecules or with other ROS to yield peroxynitrite (ONOO-), hydroxyl radical, singlet oxygen and ozone (O_3) (Hampton *et al.*, 1998). Production of nitrogen dioxide by the activity of myeloperoxidase (MPO) in the

presence of nitrite is considered a key step in the pathophysiology of LDL oxidation (Allegra *et al.*, 2007).

The observations of present study shows that co-administration of Vit.E with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly decreased the myloperoxidase activity indicating that these combinations suppressed neutrophil infiltration into the injured myocardium. The inhibition of neutrophil infiltration and its function resulting in reduced generation of oxygen free radicals, during ISO induced MI, may contribute to the protective action of these combinations against MI. Co-administration of Vit.E and LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) did not show beneficial effect on MPO activity compared to Vit.E alone. Ghule *et al.*, (2006b) reported that the fruit of *Lagenaria siceraria* shows anti-inflammatory activity in various models of inflammation.

Electrocardiograhic and biochemical findings were further confirmed by histopathological studies. Histopathological examination of myocardial tissue in control depicted clear integrity of the myocardial cell membrane. Histopathological sections of the heart injected with ISO displayed necrosis of muscle fibers with inflammatory cell infiltration, edema and fragmentation of muscle fibers which indicates involvement of oxidative stress and inflammatory processes in ISO injected myocardial injury. Treatment of Vit.E along with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO or Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly protects the myocardial injury by decreasing inflammatory process, an evidence for reduction of inflammatory cells and edema. Further, present study showed significant reduction in the MPO activity thereby neutrophil infiltration by Vit.E+GT+ISO, Vit.E+LYP+ISO and Vit.E+PGFE+ISO combinations. Co-administration of Vit.E with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) showed moderate changes in histopathological alterations.

These data further confirmed the cardioprotective action of Vit.E along with GT, LYP or PGFE in ISO injected rats.

Glycoproteins/ Glycoconjugates are important components of intracellular matrix, cell membrane and membranes of the subcellular organelles (Zachariah and Basu, 1993). Glycoconjugates are specific markers for oxidative injury of membrane components. The function of glycoproteins in stabilizing the tissue may be involved in maintaining the structural stability of collagen fibers. Significant increase in the levels of glycoprotein or glycoconjugates in serum and the heart of ISO-induced rats have already been reported (Lindberg *et al.*, 1991, Mathew *et al.*, 1982). Report shows that MI induces quantitative and qualitative changes in glycoproteins. Presence of proteins of serum origin within the extracellular matrix of damaged tissue is well established. Increase in protein bound carbohydrate complexes may be the reason for myocardial dysfunction (Das *et al.*, 1986). The observed increase in the levels of glycoproteins in ISO-injected rats may also be due to increased deposition of macromolecular components, which is a physiological adjustment to the pathological process. Judd and Wexler (1970) suggested that glycoproteins are involved in the myocardial necrosis and repair. Lysosomal membranes contain large amounts of glycoproteins which play an important role in maintaining lysosomal structure and function. These glycoproteins are considered to be the susceptible target for reactive oxygen radicals produced during hypoxic conditions (Punithavathi and Prince, 2009).

In the present study periodic acid Schiff's staining of control and experimental groups of rats was carried out. ISO injected rats shows increased expression of glycoproteins which may be due to ISO induced lipid peroxidation and diminished antioxidant status. The membrane deterioration of lysosomes by ISO-induced LPO could result in the release of more glycoproteins (Punithavathi and Prince, 2009). Treatment with Vit. E alone and its combination with GT, LYP, PGFE or LSFJ in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO, Vit.E+PGFE+ISO or

Vit.E+LSFJ+ISO) showed near normal architecture of membrane and decrease of membrane bound glycoconjugates compared to Vit.E alone treatment groups (Vit.E+ISO). The observed effects of the combinations may be due to their membrane stabilizing effects through the termination of lipid peroxidation chain reaction. Addition of GT, LYP, PGFE or LSFJ along with Vit.E shows beneficial effects rather that Vit.E alone.

Ventricular fibrosis was evidenced by Masson's trichome staining, in which heart muscle is stained in red and collagen in green. Collagen is an integral part of the normal extracellular matrix of the heart, linking the myriad of myocardial tissue components into a cohesive whole and ensuring efficient function as a biological pump (Factor and Robinson, 1988). Increased cardiac collagen synthesis and degradation of existing collagen have been observed in experimental myocardial necrosis and altered cardiac function (Takahashi *et al.*, 1990; Ravichandran and Puvanakrishnan, 1991). In the present study ISO injected rats showed increased degradation of collagen which is similar to the previous reports by Gallego *et al.*, 2002. The increased rate of newly synthesized collagen degradation observed in ISO group could be due to increase in intracellular concentration of cAMP, which might activate a specific intracellular protease or protein kinase capable of tagging collagen molecules for degradation (Schimke and Katunuma, 1975). However increased activation of collagenase, non specific neutral proteases, serine proteases (Takahashi *et al.*, 1990), lysosomal degradative enzymes, inflammatory cell releasing enzymes and plasma proteins (Weber *et al.*, 1994; Cannon *et al.*, 1983; Hansen, 1995) are some of the factors responsible for collagen degradation.

Nirmala and Puvanakrishnan (1996) showed that, increased lysosomal hydrolases and inflammatory cell proteases were responsible for collagen degradation. Inhibition of lysosomal enzymes suppresses the degradation of collagen (Berg *et al.*, 1980). The molecules which inhibit lipid peroxidation also inhibit collagen synthesis suggesting that there is an interrelationship between the two

phenomena (Nirmala *et al.*, 1999). Studies show that ISO administration increases the lysosomal hydrolases, which may be responsible for the observed collagen degradation. The same study reported that antioxidants can also prevent this degradation by inhibiting lysosomal hydrolases activity in ISO injected rats (Farvin *et al.*, 2010; Gallego *et al.*, 2002). *In vitro* experiments show that the inhibition of collagen synthesis by retinoids and curcumin could be due to their antioxidant activity (Nirmala and Puvanakrishnan, 1996; Geesin *et al.*, 1990).

Treatment of Vit.E alone and its co-administration with GT, LYP or PGFE in ISO injected rats (Vit.E+GT+ISO, Vit.E+LYP+ISO, Vit.E+PGFE+ISO) showed significant reduction of collagen degradation as compared to individual drug treatment groups. This might be due to the preventive effects of individual antioxidants and their combinations on lysosomal membrane integrity which prevents the leakage of lysosomal hydrolases from the sac and thereby prevents the disruption of collagen fibers. Further this effect may be attributed to the ability of this combination therapy to significantly attenuate ISO induced lipid peroxidation and associated oxidative stress. Vit.E in combination with LSFJ did not produce any protective effects on collagen degradation. In this context, EGCG has been reported to protect lysosomal hydrolases in ISO induced MI (Devika and Prince, 2008) and green tea polyphenols have been reported as potent intracellular inhibitors of protein kinase A (Moskaug *et al.*, 2008). This might be one of the reasons for the protecting collagen degradation by GT in the present study.

Area of infarction indicates loss of membrane integrity which might be due to significant leakage of LDH enzymes. Further increase in nitrosative stress and ROS production led to an enlarged infarct size in the ISO injected MI (Hu *et al.*, 2006). The present study showed a significant increase in % infarction in ISO injected rats. Present study also shows significant increase in ROS, LDH isoenzyme levels and nitric oxide production after ISO injection which might be the reasons for increased area of infarction in the present study. Treatment with Vit.E alone and

in combination with GT, LYP or PGFE in ISO injected rats (Vit. E+GT+ISO, Vit.E+LYP+ISO or Vit.E+PGFE+ISO) significantly decreased infarction size which might be due to their potent antioxidant activity which prevents leakage of LDH enzymes and elevated nitrosative stress. Vit.E in combination with LSFJ in ISO injected rats (Vit.E+LSFJ+ISO) slightly reduced the infarction size which was found to be non-significant.

Present study shows significant increase in caspase-3 activity and increased DNA breakdown in ISO injected rats. Increased caspase-3 activity and DNA damage indicates cardiac apoptosis and necrosis in ISO injected rats. Many studies have reported how catecholamine, especially β-adrenergic receptor (AR) stimulation, induces cardiac apoptosis or/and necrosis (Colucci *et al.*, 2002; Remondion *et al.*, 2003). In present study, we observed both apoptosis and necrosis in ISO injected rats.

It has been already reported that oxidative stress induces DNA damage (DNA fragmentation and apoptosis) (Kasai *et al.*, 1984) and addition of antioxidants inhibit DNA fragmentation and apoptosis (Verhaegen *et al.*, 1995; Galang *et al.*, 2000). Oxidation of catecholamine forms quinonoid compounds giving rise to the production of superoxide anions and subsequently hydrogen peroxide, which in the presence of iron forms highly reactive hydroxyl radicals and cause protein, lipid and DNA damage (Dhalla *et al.*, 2000). Further, increased expression of iNOS in myocardium of animals and patients with heart failure (Drexler *et al.*, 1998) may be responsible for increased numbers of apoptotic cardiomyocytes (Narula *et al.*, 1996; Olivetti *et al.*, 1997). In the present study we also observed increased nitrite production which might increase nitrosative stress followed by ISO administration; might be the reason for DNA damage and Caspase-3 activation. In this context, it has been reported that acute β-AR stimulation in ischemic heart triggered a marked increase in NO production, generated toxic peroxynitrite, activated apoptosis and eventually caused cardiac dysfunction and myocardial

injury (Li *et al.*, 2006). Chronic ISO stimulation induced an up-regulation of iNOS which produced a significant amount of NO and its toxic byproducts (peroxynitrite) in the circulation, which resulted in myocardial apoptosis by the activation of caspase-3 activity (Hu *et al.*, 2006). Calcium overload, can also activate caspases during the process of apoptosis (Green and Reed, 1998). Zhou *et al.*, (2006) have reported that antioxidants like silibinin protect rat cardiac myocytes in ISO induced DNA damage by preventing caspase-3 activation and DNA fragmentation.

Treatment with Vit.E alone and in combination with GT or LYP in ISO injected rats (Vit.E+ISO, Vit.E+GT+ISO or Vit.E+LYP+ISO) showed significant reduction in caspase-3 activity and also DNA damage. This indicates that Vit.E along with GT or LYP prevents apoptosis and necrosis in ISO injected myocardial infarction in rats. This beneficial effect of Vit.E+GT+ISO or Vit.E+LYP+ISO indicates potent antioxidant activity of these combinations which scavenge the highly toxic free radicals and prevents the DNA from damage and thereby inhibiting caspase-3 activity. In the present study, significant decrease in tissue nitrite levels by co administration of Vit.E and GT or LYP were observed, which might be one of the reasons for anti-apoptotic effects of these combinations.

In this context, Vit.E has been reported to reduce myocyte apoptosis by inhibiting the mitochondrial-dependent caspase, prevented downregulation of β-adrenergic receptor sensitivity and sarcoplasmic (SR) Ca^{2+} ATPase protein (Qin *et al.*, 2006). The antioxidants, (-) epigallocatechin gallate inhibited apoptosis in H_2O_2-exposed human umbilical vein endothelial cells (HUVEC) and cyclosporine induced nephrotoxicity by inhibiting caspase-3 activity and reducing DNA fragmentation induced by oxidants (Choi *et al.*, 2003; Shaohua *et al.*, 2003). Further LYP is reported to decrease the oxidative injury of endothelial cells induced by H_2O_2 and attenuate the expression of p53 and caspase-3 mRNA in injured cells thereby diminishing the apoptosis of injured cells (Tang *et al.*, 2009).

Treatment of Vit.E alone and co-administration with PGFE in ISO injected rats (Vit.E+ISO and Vit.E+PGFE+ISO) showed significant prevention in DNA damage. However, it did not show protective effects on caspase-3 activity suggesting that PGFE did not show anti-apoptotic effect, it prevents necrosis induced by ISO. However administration of LSFJ alone and with Vit.E in ISO injected rats (LSFJ+ISO, Vit.E+LSFJ+ISO) neither reduced DNA damage nor did caspase-3 activity compared to Vit.E alone, suggesting that LSFJ did not produce effect on apoptosis and necrosis induced by ISO.